REMARKS

Claims 1 to 38 are pending in this application.

Applicants would like to thank the Office for the withdrawal of various previously issued rejections as outlined in paragraph 2 of the Office Action.

IDS

In paragraph 3, on pages 2 and 3, the Office stated that the listing of references in the specification (pages 28 to 30) is not a proper information disclosure statement (IDS). The Office further states that unless references have been cited by the Examiner on form PTO-892, they have not been considered.

Applicants have filed a number of IDSs during the prosecution of this case including on March 11, 2005 and February 21, 2006. The Office has cited additional prior art during the prosecution.

Applicants filed a request for continued examination (RCE) in this case. According to MPEP §609.02 A.3 "Information which has been considered by the Office in the application before the filing of a RCE will be part of the file before the examiner and need not be resubmitted to have the information considered by the examiner and listed on the patent."

Accordingly, applicants respectfully request that all the information that is part of the file is considered by the Office.

Specification

In paragraph 4, the Office objected to the abstract for being too long and containing legal phraseology.

In response, applicants submit herewith an amended abstract that addresses the Office's concerns.

Also in paragraph 4, the Office objected to the specification because pages 21 to 23 contain multiple sequence disclosures that are encompassed by the definitions for nucleotides and/or amino acid sequences set forth in 37 CFR §1.821(a)(1) and (a)(2), but did not contain proper sequence identifiers.
Applicants have carefully reviewed the application and submissions with regard to sequence listings. Applicants noted that the appropriate sequence listings were previously submitted in computer readable form including an appropriate statement, but that apparently pages 21 to 23 of the specification were not amended. Applicants have now amended the specification accordingly.

In paragraph 5, on page 4, the Office objected to claim 22 as being a duplicate of claim 7.
In response, applicants have amended claim 22 to depend from claim 12.

35 U.S.C. §112 Rejections

In paragraph 6, the Office rejected claims 1 to 5, 7 to 20, and 22 to 38 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

In particular, the Office rejected claims 1 to 5, 7 to 20, 22 to 31 and 33 to 38 under 35 USC §112, second paragraph as being incomplete for omitting essential steps.

In particular the Office expressed the opinion that such a gap exists between steps (b) and (c) of these claims.

With the sole purpose of advancing the prosecution of the case and without concurring with or rejecting the Office's argument, applicants have amended the claims to include a recitation that requires that an amplification product is produced and that this product is converted into single stranded form. Support can be found on page 13, first paragraph of the specification.

For the same reason, applicants have also amended the claims to clarify the role of the anchor.

On page 5, the Office rejected the phrase "said methylated nucleotide" in claims 8, 23 and 37 for lack of antecedent basis.

In response applicants have amended the claim so that it is clearer which nucleotide of claim 1 is meant and to avoid any antecedent basis issue.

Also on page 5, the Office rejected claims 9 and 24 as indefinite in view of the recitation in (b) and (c).

In particular, the Office expressed the opinion that it is unclear if these reagents are being added to a mixture containing the sequencing primer and amplified nucleic acid molecules in single stranded form or if they are added to something else.

Also on page 5, the Office rejected claims 9 and 24 as indefinite in view of the phrases "sequential addition of all four different dNTPs" and detection of a luminescent signal" recitation in (b) and (c), respectively.

On page 6, the Office rejected claims 9 and 24 as indefinite in view of the phrase "four different dNTPs" for being unclear which four dNTPs are being used.

Also on page 6, the Office rejected claims 9 and 24 as indefinite in view of the phrase "wherein an intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide." The Office stated that the term "correlated" has not been clearly defined and there is no art recognized definition for this term and the relationship between the luminescent signal and a nucleotide being incorporated. The Office similarly rejected the phrase "wherein the intensity of said signal is indicative of the methylation status of said nucleotide." The Office stated that the phrase is confusing because it was unclear how the intensity of the signal is related to the methylation status of the nucleotide.

Applicants would like to direct the Office's attention to the fact that the highest U.S. Patent Court, the CAFC, has repeatedly stated, including as recent as June of last year (*Young v. Lumenis*, 06-1455, Fed. Cir., June 27, 2007), that claims are indefinite only when they are "not amenable to construction or are insolubly ambiguous." Also, the CAFC made clear that examination of any intrinsic evidence is required in an indefiniteness determination.

Similarly, the Manual of Patent Examining Procedure (MPEP) clarifies that all that is required for a particular phrase to be definite is a reasonable degree of clarity and particularity. The MPEP also emphasizes that the definiteness of claim language must

be analyzed, not in a vacuum, but in light of: (A) The content of the particular application disclosure; (B) The teachings of the prior art; and (C) The claim interpretation that would be given by those possessing the ordinary level of skill in the pertinent art at the time the invention was made (MPEP §2173.02). The MPEP refers to *Metabolite Labs* in which the CAFC stated that only when a claim remains insolubly ambiguous without a discernible meaning after all reasonable attempts at construction must a court declare it indefinite. *Metabolite Labs., Inc. v. Lab. Corp. of Am. Holdings*, 370 F.3d 1354, 1366 (Fed. Cir. 2004).

Applicant submits that the present claims readily meet the threshold requirement of clarity and particularity.

In this context, applicants also note that MPEP §706.07 emphasizes the equities involved in the patent prosecution process. Applicants submit that the importance of these equities become even more pressing in view of the fact that applicants may be limited in the future in their ability to file RCEs and/or continuations. Many of the objection and rejections made in this Office Action could have been made in one of the previous Office Actions, giving applicants the chance to resolve these issues before filing an RCE.

To advance the prosecution of this case, applicants have amended the phrase "four different dNTPs" to "dATP, dCTP, dTTP and dGTP" based on page 14 of the specification [Four kinds of dNTP (i.e., dATP, dCTP, dTTP and dGTP . . .].

For many of the indefiniteness rejections issued above applicants would like to direct the Office to the specification as a whole which answers many of the questions posed. For example, the background section and the art cited therein sheds light on the questions posed by the Office with regard to the terms "correlated" and "indicative." The art cited therein in particular will provide the Office with a sample of the teachings of the prior art and the claim interpretation that would be given by those possessing the ordinary level of skill in the pertinent art at the time the invention was made (MPEP §2173.02). The further clarification requested by the Office regarding (b), (c) and (d) of claims 9 and 24, also can be readily resolved by considering the claims in the context prescribed by MPEP §2173.02. However, the Office is urged to make any specific suggestions as to how to amend the claims in accordance with MPEP §2173.02.

With respect to the use of the term "correlated," the Office may find claim 1 of U.S. Patent 7,198,939 instructive, which issued on April 3, 2007 in Art Unit 1634, in particular when considered in view of its specification (see, col. 15, line 4).

With regard to the term "indicative," the Office may find claim 1 of U.S. Patent 7,270,957 instructive, which issued on September 18, 2007 in Art Unit 1634, in particular when considered in view of its specification (see, col. 65, line 55).

On pages 6 and 7, the Office rejected claims 12 to 20, 23 to 26, 28 to 29 and 38 as indefinite as not clearly setting forth a step for diagnosing a pathological condition or providing a prognosis for a pathological condition. The Office also rejected the claims for being unclear how detecting a methylated nucleotide sequence allows for the diagnosis and/or prognosis of a pathological condition.

The standards for indefiniteness have been discussed above. Applicants submit also in the context of the present rejection that the threshold requirements of clarity and precision have been readily met. However, in an attempt to further the prosecution of this case, applicants have further amended the claims. However, if the change is not satisfactory to the Office the Office is urged to suggest claim language that the Office believes would improve the clarity and precision of the claims in accordance with MPEP §2173.02, which states in this context: "Examiners are encouraged to suggest claim language to applicants to improve the clarity or precision of the language used, but should not reject claims or insist on their own preferences if other modes of expression selected by applicants satisfy the statutory requirement."

35 U.S.C. §103 Rejection

Prior to arguing the specific rejections under 35 USC §103(a), applicants would like to make the following observations:

As the discussion in the background section indicates, a lot of research was conducted with regard to the methylation status of nucleotides starting in the late nineties, in particular after its significance in tumor development was recognized. As also described in the background section, many of these methods disclose bisulfite-treatment of the DNA of interest and subsequent assessment of the methylation status of nucleotides. Two references of this type have been discussed in detail in the previous

three responses. A further of these references is discussed herein. As before, applicants will show herein that, also the inventors' own Uhlmann '99 reference, which discloses bisulfite-treatment of an DNA of interest and subsequent assessment of the methylation status of the nucleotides, does not render the invention obvious in view of Nyren (2001).

However, in view of the abundance of work published that contains disclosure similar to the disclosure of the three primary references so far discussed during the prosecution of this case, applicants would like the Office to take note of the fact that while Nyren's method was available as early as 1998 (see, e.g., PCT publication, but also other Publication such as in Science), it was not employed for determining the methylation status of nucleotides as claimed in the present invention until the inventors suggested to do so.

Since then the presently claimed method has been widely adopted in the industry and has been considered the **"Gold Standard" for quantitative methylation analysis**. (See attached Biotage May 2006 Newsletter, e.g., last page). See also Biotage Annual Report 2004, p. 13: "It offers several advantages that other techniques do not offer. We basically get a much more detailed read-out profile of what might be going on in a given cancer. To put it simply, **it's like using a telescope to look at the stars instead of using a pair of binoculars"** Ralf Krahe, Associated Professor, Department of Molecular Genetics at M.D. Anderson Cancer Center.

The Office is in this context directed to Fed. Reg. Vol. 72, No, 195, p 57534, right col. to 57535, left col. (**Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in *KSR International Co.* v. *Teleflex Inc.*)**.

However, irrespective of the above "secondary considerations", applicants will show below that no *prima facie* case of obviousness has been established by the Office. Applicants will discuss a number of reasons that support non-obviousness and requests that each of those reasons is considered separately by the Office.

In paragraph 7 and 8, on pages 7 to 15 the Office rejected claims 1 to 5, 7 to 9, 11 to 12, 19 to 20, 22 to 24, 26 to 33 and 36 to 37 under 35 USC §103(a) as being obvious over Uhlmann et al., (Electrophoresis, 1999) (hereinafter "Uhlmann '99")) in view of U.S. Patent 6,258,568 to Nyren et al. (hereinafter "Nyren").

The Office expresses the opinion that Uhlmann'99 teaches a method for identifying methyl cytosines comprising treating a sample containing genomic DNA with sodium bisulfite and amplifying the sample by PCR. The Office states that Uhlmann '99 teaches that the amplified nucleic acids were sequenced by the dideoxynucleotide chain termination method to determine the methylation state of the amplified product.

The Office conceded that Uhlmann does not teach a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules.

The Office also acknowledged that Uhlmann does not teach that the amplified nucleic acids were sequenced using a real-time sequencing method.

However, the Office expressed the opinion that these teachings are provided by Nyren.

In particular, the Office expressed the opinion that Nyren teaches that one or more of its PCR primers may carry a functional group such as a biotin which permits subsequent immobilization (col. 8, lines 22 to 31) and that real-time sequencing adds a wide variety of desirable advantages rendering the invention as claimed obvious.

The Office concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann '99 by using pyrosequencing to determine the sequence of the amplified DNA fragment as suggested by Nyren.

As the Office noted, Uhlmann 99's amplification primers are not detectably labeled. Uhlmann's amplification product is cloned to produce single stranded DNA. This cloning, as in references discussed in previous communications with the Office, follows Unhmann 99's amplification and precedes the sequencing. ("[P]lasmid DNA of positive clones . . . were sequenced by the dideoxynucleotide chain-termination method." (see 2.5, pages 1751 and 1752)).
Claim 1 recites:

(b) amplifying said nucleic acid molecule . . . via at least one amplification primer . . . detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules to generate a single stranded amplified nucleic acid. . . *[emphasis added]*

Claim 12 contains similar language.

Applicants submit that the person skilled in the art would be reluctant to make the modification to Uhlmann that the Office suggested, namely detectably label Uhlmann 99's amplification primers as it would interfere with the subsequent cloning step. For example, U.S. Patent 6,589,736 to Rothschild et al. discloses in its background section, "PCR products that are biotinylated are not suitable material for cloning." (col. 7, starting on line 23). The same patent states also in col. 34, starting on line 40 that "the presence of biotin on the nascent DNA can interfere with its subsequent utilization in cloning or hybridization analysis."

Thus, the modification proposed by the Office would render Uhlmann 99 unsatisfactory for its intended purpose (see MPEP §2143.01, V.)

Further technical difficulties (Fed. Reg. Vol. 72, No, 195, p 57534, right column) associated with using biotinylated primers are also noted in the exchange between John Dixon and Karl Voss (1996) attached hereto ("Biotin-PCR-primers").

Other technical difficulties flowing from the suggested combination of Uhlmann '99 and Nyren could have been expected to flow from the complexity of the reaction mixture used for pyrosequencing. This mixture is considerably more complex that the dideoxynucleotide chain termination sequencing mixture used, e.g., in Uhlmann '99. The effects of a chemical modification of the DNA as used in Uhlmann '99 and any residual chemicals were unclear at the time the invention was made.

In Uhlmann '99, the amplification product is cloned. In contrast to Eads et al. (see discussion in response to the Office Action of November 15, 2006), the single stranded amplified nucleic acid molecule is present for real time sequencing in (c).

With regard to the Office's statement regarding the preamble of claim 12, applicants would like to point out that subsection (d), irrespective of the weight given to the preamble of the claim, is part of this claim and should be considered by the Office. As the MPEP §2143.03 points out, a claim limitation should be considered even if the Office believes such a limitation indefinite ("[A]ll the limitations of the claims must be considered and given weight" *[emphasis added]*). Applicants can find no indication on pages 10 to 13 of the Office Action how this claim limitation is made obvious by the combination of Uhlmann '99 and Nyren to support a *prima facie* case of obviousness and notes the Office's statement made in context of the discussion of dependent claims 13 to 16, 18 and 38 on page 16, third paragraph.

Similarly, with regard to the Office's statement regarding the preamble of claim 32, applicants can not find any indication on pages 13 to 15 of this Office Action, how the

claim limitation set forth under (e) of this claim is made obvious by a combination of Uhlmann '99 and Nyren to support a *prima facie* case of obviousness.

In paragraph 9, on pages 16 to 17, the Office rejected claims 13 to 16, 18 and 38 under 35 USC §103(a) as being obvious over Uhlmann '99 in view of Nyren as applied to claim 12 above, and in further view of U.S. Patent 5,786,146 to Herman.

In paragraph 10, on pages 17 and 18, the Office rejected claim 17 under 35 USC §103(a) as being obvious over Uhlmann '99 in view of Nyren and Hermann as applied to claim 12 and 38 above, and in further view of U.S. Patent Pub. US2003/0232351 to Feinberg.

The deficiencies of Uhlmann '99 and Nyren have been discussed above. Applicants respectfully submit that neither Hermann nor Feinberg alleviate these deficiencies.

In paragraph 11, on pages 18 and 19, the Office rejected claims 10, 25, and 34 under 35 USC §103(a) as being obvious over Uhlmann '99 in view of Nyren as applied to claims 1 and 12 above, and further view of U.S. Patent 7,078,168 to Sylvan.

The deficiencies of Uhlmann '99 and Nyren have been discussed above. Applicants respectfully submit that Sylvan does not alleviate these deficiencies

Applicants respectfully submit that Sylvan's Fig. 4(a) needs to be considered in conjunction with Fig. 6. Here the expected allele frequencies are plotted against the obtained allele frequencies. As can be seen in Fig. 6, at an expected allele frequency of 5% the actually obtained allele frequency ranges from 6% to 11%.

The Office is in this context also directed towards new claim 39. Support for new claim 39 can be found in the paragraph bridging pages 23 and 24.

The undersigned sincerely urges the Office to call her at the number provided below to discuss any issues that might arise in the further prosecution of this case.

The fee for one additional claim in excess of 20 and a three months extension of time is submitted herewith. However, the Commissioner is authorized to charge or credit deposit account no. 50-3135 as required.

Respectfully submitted,

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